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(12) **UK Patent Application** (19) **GB** (11) **2 268 187** (13) **A**

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GB 2217346 A EP 0356785 A1 EP 0264484 A1
WO 83/01581A1 US 4908315 A US 4839292 A
US 4435508 A US 4416993 A US 4142940 A

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(54) **Cell culture vessels**

(57) **A vessel suitable for culturing cells has part of the vessel replaced by a gas-permeable membrane.**

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1990.

GB 2 268 187 A

1/1

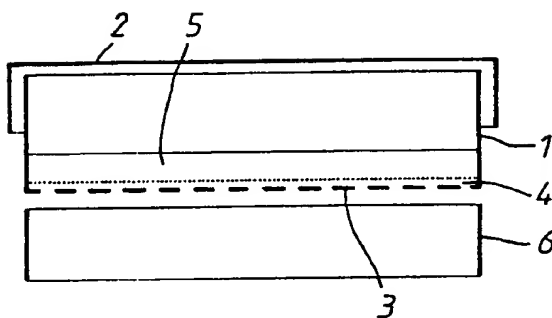


Fig.1

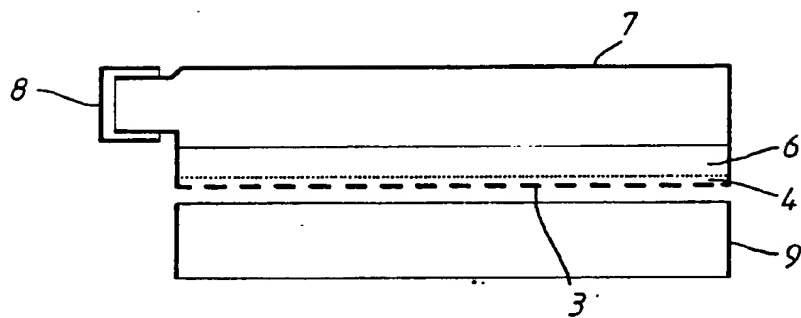


Fig.2

IMPROVEMENTS IN AND RELATING TO TISSUE CULTURE VESSELS

The present invention relates to vessels suitable for cell culture, in particular it relates to culture vessels modified to improve the supply of oxygen to the cells being cultured therein.

Perspex or glass petri-dishes and screw-top culture flasks are commonly used for animal cell tissue culture. The culture of animal cells is very dependent upon the ability to supply sufficient oxygen to the cells without causing cellular damage. The supply of oxygen for cell respiration is from the atmosphere in the header space above the cells via the liquid culture medium. Such oxygen is only sparingly soluble in culture media ($7.6 \mu\text{g/ml}$ at 30°C) and diffusion in liquid is relatively slow ($D=2.7 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$ at 30°C) and hence the depth of the liquid culture medium is crucial. For example, for human epidermis cell culture the depth of medium should not exceed 2-3mm, since greater depths could seriously limit the oxygen supply to the cells.

Aeration of the culture, by for example, sparging, surface aeration, medium perfusion, can increase the oxygen availability, however such methods can cause cellular damage. Silicone rubber tubing has been used to improve

gas exchange in cell suspension culture (see Freshney, R.I., 1986, Animal Cell Culture. . . Practical Approach Series, IRL Press Oxford/Washington. p.42) but this method is expensive and inconvenient to use.

It has now been found that, by replacing part of the culture vessel with a gas-permeable membrane, the diffusion of oxygen to the cells being cultured is greatly improved.

According to one aspect of the invention there is provided a vessel suitable for culturing cells wherein part of the vessel is replaced by a gas-permeable membrane.

Vessels commonly used for tissue culture include culture dishes, such as petri-dishes, and flasks, such as flat-bottomed Roux bottles. Such culture vessels are usually made of glass, plastic or perspex. In the case of petri-dishes the base of the dish may be replaced by a gas-permeable membrane, whereas with the Roux bottle it is the flat side wall upon which the bottle is usually rested, which may be replaced by the gas-permeable membrane.

The gas-permeable membrane may be made of silicone rubber, which is five times more permeable than water to oxygen

and therefore offers much less resistance to the diffusion of oxygen than conventional depths of culture medium. Such a membrane may have a thickness of from 0.05 to 0.2mm. Even a comparatively thick membrane (0.2mm) will allow the diffusion of fifty times more oxygen to cells compared to a 2mm depth of nutrient medium.

Alternatively the membrane may be made of fluoro-ethylene-propylene copolymer (FEP-Teflon) or other gas-permeable polymer that is not deleterious to cells.

The invention will be further described by way of reference to the following drawings in which:-

Figure 1 is a vertical section through a petri-dish with its base replaced by a gas-permeable membrane

Figure 2 is a vertical section through a Roux bottle with its flat "bottom" replaced by a gas-permeable membrane.

In Figure 1 the petri-dish 1, which is made of glass, has a lid 2. The bottom of the petri-dish 1 has been replaced by an oxygen-permeable membrane 3 made of silicone rubber, which has been attached to the petri-dish using an appropriate adhesive (silicone rubber). Lying on top of the membrane 3 is a layer of cells 4, which in turn are

overlaid by nutrient medium 5. The petri-dish 1 rests on a supporting ring 6 which permits air access to the membrane.

In Figure 2 the Roux bottle 7, which is made of glass or Perspex, has a screw top 8. The flat bottom, which is effectively a side wall of the flask, has been replaced by a silicone rubber membrane 3, which has been attached using an appropriate adhesive (silicone rubber). Lying on top of the membrane is a layer of cells 4, such as keratinocytes, which in turn are overlaid with an appropriate nutrient or growth medium 6. The bottle 7 rests on a support 9 which permits air access to the membrane 3.

Using the culture vessels modified according to the invention allows greater depths of culture medium to be used. The vessel with the membrane attached, and optionally containing culture medium, can be sterilized by appropriate conventional methods such as autoclaving, ultra violet radiation, ethene oxide etc.

The modified culture vessels of the present invention could be particularly advantageous when culturing layers of cells, such as in skin cultures where it is desirable to produce layers of keratinocytes for skin grafting. The

gas-permeable membrane allows the passage of oxygen directly to the dividing cells which lie on the membrane surface. In the conventional method of growing keratinocytes the supply of oxygen to the dividing cells is further curtailed by the overlying cell layers.

Another possible advantage of growing keratinocytes for skin grafting in a vessel according to the invention is that it is possible to cut out from the flask or dish the membrane, with the cultured cell layer attached, and transfer it directly to the area of the patient to be grafted. This would avoid the difficulty of removing the cultured tissue from the surface of the vessel, with the consequent damage to the cells. It would also makes it easier to handle the cultured tissue. The cultured tissue may also be sectioned on the membrane.

Yet another possible advantage of modifying a culture vessel, in accordance with the invention, is that such vessels could be used for initial sample bottles, for example, for amniocentesis. As a result of the membrane the sample of cells would be supplied with extra oxygen prior to being cultured, which might greatly improve cell survival.

CLAIMS

1. A vessel suitable for culturing cells wherein part of the vessel comprises a gas-permeable membrane.
2. A vessel as claimed in claim 1, wherein the gas-permeable membrane is made of silicone rubber.
3. A vessel as claimed in claim 1, wherein the gas-permeable membrane is made from fluoro-ethylene propylene co-polymer.
4. A vessel as claimed in claim 1, wherein the gas-permeable membrane is made from a gas-permeable polymer that is not deleterious to cells.
5. A vessel as claimed in any one of claims 1 to 4, wherein the membrane has a thickness in the range from 0.05 mm to 0.2 mm.
6. A petri dish for culturing cells, wherein the base of the dish comprises a gas-permeable membrane.
7. A Roux bottle for culturing cells wherein a flat side wall of the bottle comprises a gas-permeable membrane.

8. A method of culturing cells comprising,
providing a culture vessel having a wall made from a
gas-permeable membrane,
depositing a layer of cells on the membrane within
the culture vessel, and
overlaying the layer of cells with a growth medium.
9. A method as claimed in claim 8, including
ventilating the membrane by permitting air access thereto.
10. A method as claimed in claim 8 or claim 9, including
the additional step of removing the membrane from the
vessel with the cultured cell layer attached.
11. A vessel suitable for culturing cells substantially
as herein described with reference to Figure 1 or Figure 2
of the accompanying drawings.
12. A method of culturing cells substantially as herein
described.

- 8 -

Patents Act 1977
Examiner's report to the Comptroller under
Section 17 (The Search Report)

Application number
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Relevant Technical fields

(i) UK CI (Edition) Contd. from page 1

(ii) Int CI (Edition)

Databases (see over)

(i) UK Patent Office

(ii)

Search Examiner

C SHERRINGTON

Date of Search

1 SEPTEMBER 1993

Documents considered relevant following a search in respect of claims

Category (see over)	Identity of document and relevant passages		Relevant to claim(s)
X	US 4839292	(JOSEPH G CREMONESE) - whole document	1,4 at least
X	US 4908315	(AGRISTAR, INC) - whole document	1,4 at least

575

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

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